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Wastewater aerosols produced during flushing toilets, WWTPs, and irrigation with reclaimed municipal wastewater as indirect exposure to SARS-CoV-2

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ARTICLE INFO

Editor: Teik Thy Lim

Keywords:

SARS-CoV-2
COVID-19
Bioaerosol
Aerosolized wastewater
Wastewater
Virus-laden aerosols

ABSTRACT

The detection of SARS-CoV-2 RNA in raw and treated wastewater can open up a fresh perspective to waterborne and aerosolized wastewater as a new transmission route of SARS-CoV-2 RNA during the current pandemic. The aim of this paper is to discuss the potential transmission of SARS-CoV-2 RNA from wastewater aerosols formed during toilet flushing, plumbing failure, wastewater treatment plants, and municipal wastewater reuse for irrigation. Moreover, how these aerosols might increase the risk of exposure to this novel coronavirus (SARS-CoV-2 RNA). This article supplies a review of the literature on the presence of SARS-CoV-2 RNA in untreated wastewater, as well as the fate and stability of SARS-CoV-2 RNA in wastewater. We also reviewed the existing literatures on generation and transmission of aerosolized wastewater through flush a toilet, house's plumbing networks, WWTPs, wastewater reuse for irrigation of agricultural areas. Finally, the article briefly studies the potential risk of infection with exposure to the fecal bioaerosols of SARS-CoV-2 RNA for the people who might be exposed through flushing toilets or faulty building plumbing systems, operators/workers in wastewater treatment plants, and workers of fields irrigated with treated wastewater – based on current knowledge. Although this review highlights the indirect transmission of SARS-CoV-2 RNA through wastewater aerosols, no research has yet clearly demonstrated the role of aerosolized wastewater in disease transmission regarding the continuation of this pandemic. Therefore, there is a need for additional studies on wastewater aerosols in transmission of COVID-19.

1. Introduction

In light of the emergence of the new coronavirus in China caused worldwide pandemics, it seems necessary to have a greater understanding of the role of wastewater as a risk factor for public health. Emerging pathogens from humans can enter sewage systems. It could be because feces and urine from humans can contain a wide range of pathogens, which sewage systems can transport to wastewater treatment plants [1,2].

The SARS-CoV-2 is enveloped RNA virus of the viral family Coronaviridae like SARS-CoV and MERS-CoV, which can cause respiratory illnesses known as COVID-19 [3–5]. Reviewing previous studies on SARS-CoV and MERS-CoV revealed that their viral RNA was detected in

feces [6–8]. Likewise, during the current outbreak of COVID-19, evidence suggests that SARS-CoV-2 RNA can be found in sewage due to body excretions (saliva, sputum, urine, and feces) [4,9–12]. Since the presence of SARS-CoV-2 RNA in wastewater was verified, this topic has sparked increased interest in detecting SARS-CoV-2 RNA in wastewater [2,13–17]. According to recently published studies, viral loads of SARS-CoV-2 RNA have been reported in influents and effluents of wastewater treatment plants [12,18–26]. A wide range of concentration methods was applied to detect SARS-CoV-2 RNA in wastewater like ultrafiltration [27,28], ultracentrifugation [29], cell culturing [30,31], PEG precipitation [24,32,33], aluminum hydroxide adsorption-precipitation method [34], viral nucleocapsid staining [35], and electronegative membrane adsorption followed by direct RNA

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<https://doi.org/10.1016/j.jece.2021.106201>

Received 13 June 2021; Received in revised form 5 August 2021; Accepted 10 August 2021

Available online 13 August 2021

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extraction [33,36]. Despite this, PCR-based technologies (e.g., RT-qPCR, digital PCR) are the most widespread detection method for SARS-CoV-2 RNA in fecal samples [37–41].

Although currently, available evidence does not confirm SARS-CoV-2 transmission through sewage or wastewater systems, the experts of the University of Stirling have warned that it is not a time to neglect the potential spread of SARS-CoV-2 RNA via sewage to protect public health. The authors also suggested that the transmission of SARS-CoV-2 RNA in untreated sewage might increase the potential of virus aerosol droplets, especially at faulty plumbing in buildings or hospitals, sewage pumping stations, uncovered aerobic wastewater treatment facilities, and near waterways that are receiving wastewater [42]. In a prior investigation by McKinney et al., wastewater was found to seem partially responsible for a previous SARS outbreak because of a defective ventilation and plumbing system [43]. A publication made by Yuan et al. points that sewage could be considered as the potential COVID-19 spread in Guanzhou, China [44]. Therefore, according to the similarities between SARS-CoV-2 and SARS-CoV-1, sewage and sewerage system can pose SARS-CoV-2 transmission risk.

In fact, the detection of SARS-CoV-2 viral material in sewage currently warns against the potential exposure and transmission of SARS-CoV-2 RNA through sanitation systems as well as wastewater treatment plants. This is particularly critical due to the prolonged pandemic time and the increase of viral genome loads in untreated sewage, followed by the rise in the number of infected people with COVID-19 [45,46].

As presented, most published data have focused on detecting SARS-CoV-2 RNA in patients' feces or untreated wastewater. There is a significant knowledge gap about water droplets of SARS-CoV-2 from sewage. It seems that SARS-CoV-2 viral material, which may be transmitted via wastewater systems, might increase the risk of exposure to SARS-CoV-2 and potentially severe health consequence [41,47]. The scale of the potential spread of SARS-CoV-2 RNA through wastewater collection systems and sewage treatment plants is unknown due to a lack of testing [42,47].

Hence, further research is required to assess SARS-CoV-2 in aerosolized wastewater. In this work, peer-reviewed articles were searched for papers relevant to the production and transfer of wastewater aerosols during toilet flushing, building plumbing networks, and wastewater treatment processes. Then, we summarized the existing literature on SARS-CoV-2 fate and stability in the raw wastewater and treated wastewater, aerosolized SARS-CoV-2 from toilets within WWTPS, and irrigation of treated municipal wastewater. We also discuss the risk of exposure to SARS-CoV-2-contaminated aerosols of wastewater for exposed people.

2. Fate and stability of SARS-CoV-2 RNA in wastewater

The presence of SARS-CoV-2 RNA in sewage raises the need for further information about its fate in wastewater and wastewater treatment plants; there are still few studies on it. This is mainly because recent studies have focused on detecting SARS-CoV-2 RNA in raw wastewater without investigating factors affecting this virus's survival in wastewater. Therefore, our information on these factors is greatly restricted to what we know about other CoVs because of a lack of prior research on factors affecting SARS-CoV-2's ability to survive in sewage. Currently, several factors have been presented by the authors on influencing coronaviruses' survival in wastewater, including viral structure, wastewater characteristics/composition, temperature, and pH [48–54]. In recent years, numerous studies were carried out to investigate the presence and fate of a wide range of non-enveloped enteric viruses in wastewater because enveloped viruses are often assumed to be present at low levels and undergo fast deactivation in aqueous environments [14,55,56].

Nevertheless, enveloped viruses have been implicated in the most deadly pandemics and outbreaks such as influenza, SARS, MERS in the

20th and 21st Centuries [56]. Despite knowing little about the fate and survival of enveloped viruses in domestic sewage, some evidence revealed that the idea of viral envelope's absence in sewage is not always accurate. For instance, reviewing current knowledge about avian influenza viruses promotes this virus's long-term survival in some aqueous environments [10].

Similarly, a mutated human influenza virus can persist in water for long periods [57]. According to this information, sewage can act as a vector for transporting some enveloped viruses. It should also be considered that these enveloped viruses could survive sufficient time to reach wastewater treatment facilities, and therefore, they cannot be eliminated in wastewater systems [56].

The survivability of coronavirus is influenced by viral structure. The lifespan of enveloped viruses such as coronavirus is shorter than non-enveloped viruses. The reason would be the activity of detergents and proteolytic enzymes on the virus's outer layer of lipid envelope [48,50].

Wastewater characteristics and the compounds available in wastewater can play a crucial role in the survival and inactivation of coronaviruses in sewage. Coronavirus can be quickly inactivated in wastewater due to the existence of chemical which has the anti-viral properties. Studying virus survival in wastewater has also shown that organic matter and suspended solids in wastewater may have a strong influence as helper contributions on the survival of viruses in sewage because they can afford protection to viruses which may result from adhering to these particles [54,55]. More details of studies factor are tabulated in (Table S1).

Evidence from recent studies suggests that temperature has the most crucial effect on SARS-CoVs survival in wastewater [58,59]. The result of these studies has also indicated that the persistence of SARS-CoVs in sewage reduces with an increase in temperature. Thus, SARS-CoVs can survive and remain infectious for long periods at low temperatures in wastewater (Table S2) [50,60]. Likewise, preliminary research on SARS-CoV-2 illustrated that low survival times of SARS-CoV-2 RNA in sewage occur in temperatures more than 20 °C [61].

The fecal pH has a significant effect on the survival of SARS-CoV-1 starting from three hours in newborn baby's stools, which is slightly acidic, to four days at pH up to 9 in diarrhea stool of grown-ups. Conversely, results from the initial research on SARS-CoV-2 have shown that SARS-CoV-2 in suspension does not considerably decrease following titration (60 min) at various pHs (3–10) [54,62].

Above all, it should be noted that amount of SARS-CoVs in wastewater may change seasonally and daily according to wide-ranging factors, including the prevalence of disease during outbreaks in communities and the rate of viral shedding (i.e., feces, urine, vomit), which discharges into the wastewater system and meteorological and environmental conditions. The temperature of wastewater changes seasonally because seasonal variation in weather and soil temperature impacts the heat transfer between wastewater and the surrounding ambient [14].

Based on data from different studies, SARS-CoV-1 and MERS-CoV can maintain viability under various ecological conditions in wastewater. In view of the evidence, the survival period of SARS-CoV-1 in sewage is 14 days at 4 °C or 2 days at 20 °C, which is considerably related to numerous environmental factors (such as temperature, solar, or UV inactivation) and their association with biofilms. Furthermore, its RNA could still be detected in the sewage after 8 days, even if the virus was inactive [63,64]. Similarly, a recent analysis of the wastewater in Arizona, USA for SARS-CoV-2 RNA revealed that at 20 °C, the remains of virus in sewage is estimated to be at least 25%, even in a circumstance where the average time of travel of sewage through the sewer network is 10 h and the stability of virus is reasonably low [65].

3. SARS-CoV-2 in aerosolized wastewater

Aerosolized viruses, which may occur due to entering viruses into sewerage and settling on particles of fecal matter, can be regarded as one

of the potential transport pathways for airborne spread diseases. With regard to current evidence, fecal aerosols generating from virus-rich excreta in sewage and drainage systems may be possible to enter buildings as a result of strong upwards airflows, inadequate traps, and non-functional water seals [66–69], and fecal aerosols seem to be formed during the wastewater treatment processes on a large scale. Further, aerosol transmissibility of viruses is likely to be feasible during irrigation by treated wastewater because a conventional WWTP without disinfection can usually provide a substantial reduction of viruses but not complete removal. This issue becomes even more important if high viral loads in the influent wastewater arrive at WWTPs during pandemics, which could cause the inadequate decrease of viruses before discharge [54]. In research, Ali et al. investigated the reduction of SARS-CoV-2 RNA concentration in different sectors of two WWTPs in Israel. Their findings highlight the potential and shortcomings of traditional wastewater treatment in lowering SARS-CoV-2 RNA concentrations, as well as early evidence for the necessity of tertiary treatment and disinfection by chlorine in preventing viral spread to the environmental surroundings [70].

It is noteworthy that fecal bioaerosols can consist of viable and dead pathogens, which can pose a severe risk to public health, especially exposed people (e.g., residents of buildings, workers of sewage treatment plants, or residents of neighboring areas, and farmers) [71]. Observations to date indicate that microbial bioaerosols exposure has been connected to a wide range of health effects involving infectious diseases, acute toxic effects, allergies, and cancer [72,73]. Interestingly, these negative health effects of fecal bioaerosols on humans come from their size, which can easily be transmitted by inhalation, ingestion, and skin contact. Therefore, they are usually capable of causing infections among people [74–76].

Recent pandemics in the 20th century led to increased awareness of exposure risks to fecal aerosols/droplets, including emerging pathogens, and release during sewage collection and treatment systems. To date, although the role of fecal bioaerosols in the transmission of SARS-CoV-2 remains uncertain, it seems to be debating whether SARS-CoV-2 is possible to transmit through fecal bioaerosols or not. To this end, the authors have proposed studying the possibility of fecal bioaerosols as a route of transmission for SARS-CoV-2 in the prevention and proliferation of a recent pandemic [77].

Based on the prior literature, during the SARS-CoV-1 outbreak in Hong Kong (2003), wastewater-associated bioaerosols were reported as a key mechanism of SARS-CoV-1 transmission among individual homes and buildings [66,78]. On balance, authors stated that the survival of the virus in sewage due to the lack of adequate disinfection could increase contagion risk [79]. For instance, Yu's study group, in 2003, illustrated that fecal aerosols, which had been transmitted via sewer systems, were involved in the spread of the virus of SARS-CoV to more than 300 residents in the Amoy Gardens outbreak [80]. It should be mentioned that SARS-CoV concentrations in sewage and their dissemination potential are directly affected by the number of households connected to the urban wastewater collecting system [81,82].

Consequently, according to the similarities between SARS-CoV-1 and SARS-CoV-2, it is suspected that the SARS-CoV-2 RNA can be transmitted through sewage aerosolization in the current outbreak [54,83]. A recent study by Gormley et al. explained that the wastewater system, in some circumstances, can enable airborne transmission of SARS-CoV-2 [84]. Similarly, McDermott's research group confirmed that fecal bioaerosols are among the significant routes transmitting SARS-CoV-2 in hospitals [85]. This result was in line with findings reported by Liu et al., indicating the high concentration of airborne SARS-CoV-2 inside the patient mobile toilet room Fangcang Hospital due to aerosolization from feces and urine [86]. Later, further information was given by Xu et al. that the sewage system was suggested as one possible transmission route of SARS-CoV-2 in the Diamond Princess Cruise ship [87]. Furthermore, Zhang et al. considered three hospitals in Wuhan and detected SARS-CoV-2 RNA in the form of aerosol with a concentration of

(285–1130 copies/m³). Their results indicated a substantial viral spill-over in the hospital outside settings, which might have been produced by infected people exhalation or bioaerosols from wastewater that carries SARS-CoV-2 RNA [88]. Another point in which EC et al. reported research on SARS-CoV-2 transmission in flight, covering 130 individual flights and two studies on airplane wastewater. PCR-positive SARS-CoV-2 samples were found in two wastewater investigations, but with Cycle threshold values (C_t) varying from 36 to 40 [89].

Thus, at the same time, when transmission routes of novel coronavirus are attracted the world's attention, the chance of spreading SARS-CoV-2 via the fecal-oral route must not be ignored [45]. There is no doubt that the continuous pandemic of COVID-19 emphasizes the need for more excellent knowledge associated with transmission routes through wastewater exposure pathways; however, fecal bioaerosol is another route of SARS-CoV-2 transmission that has been less investigated [83].

4. SARS-CoV-2 aerosols spray from toilets

4.1. Toilet plume aerosols created by toilet flushing

Based on the flushing analysis process, by pressing the flushing button, a water volume goes into the toilet bowl from its boundary wall when the others sprinkle over the bowl with a great flow velocity, so at the same time, the phenomenon of siphon happens and causes to generate the contaminated fine droplets. Furthermore, the siphon phenomenon also leads to the increase in fluid pressure and weight of blended liquid. The sprinkling fluid, which cleans the toilet bowl wall, causes the formation of the near-wall vortices. The force of inertia frequently leads to moving upward of the vortices, resulting in an upward vortex flow of air in the bowl of the toilet. This process drives bioaerosol emissions from the toilet bowl into the toilet's surrounding air and eventually spreads them (Fig. 1). Hence, as can be seen from several studies, the generation of bioaerosols during flushing a toilet is likely to be affected by the numerous interactions between air and liquid, the flushing ways, and toilet structures (Fig. 2) [90,91].

The relationship between inhalable airborne particles generated from disturbed sewage and infection spread has been suggested for a

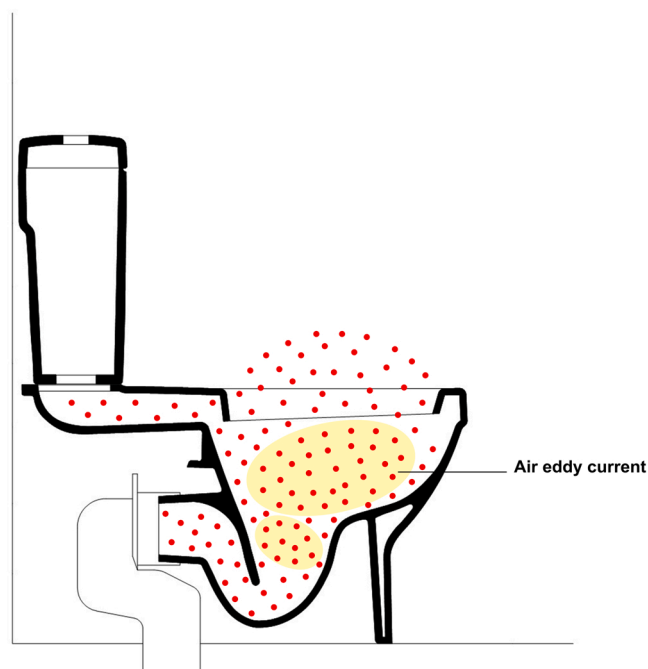


Fig. 1. The generation of air eddy current in the toilet bowl in the time of flushing [92].

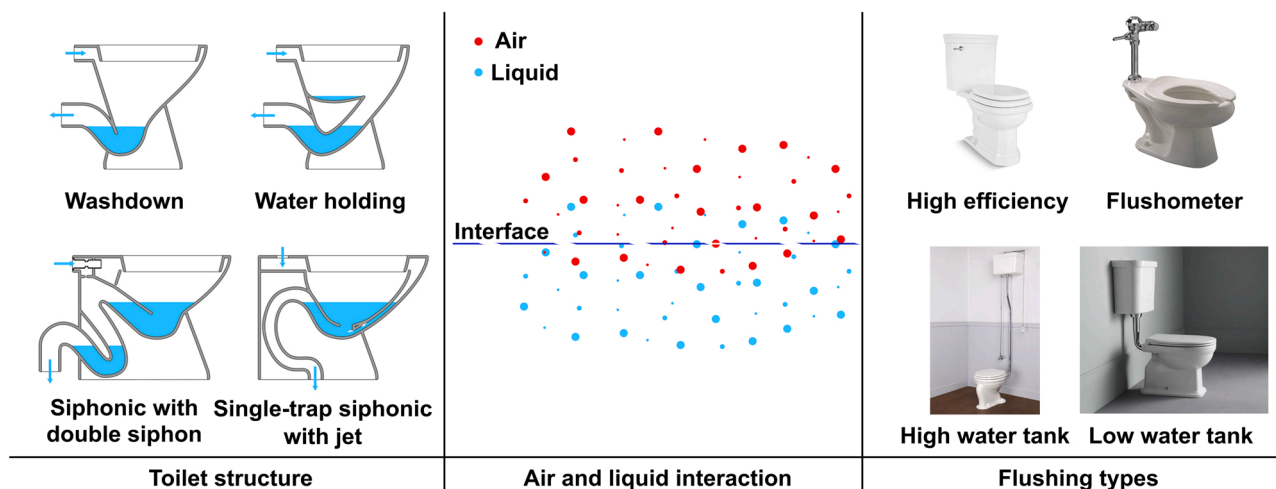


Fig. 2. Parameters which affect bioaerosol generation during toilet flushing.

century. Therefore, scientists have formerly been concerned that toilet plumes pose a risk for transmitting infectious diseases because a large number of bioaerosols can be generated by the high pressure and turbulence of toilet flushing [90]. Nevertheless, a few research studies have been dedicated to this issue [80,93]. A recent study by Li et al. was conducted on the effects of toilet flushing on virus transmission promotion. The results indicated a high fluctuation in both types of toilets (single-inlet flushing and annular flushing) tends to create a 5 m/s upward velocity that can throw the aerosol away from the toilet bowl. Besides, they observed that 40–60% of aerosols rose from the toilet seat to a height of 106.5 cm above the floor [91]. Another study by Knowlton et al. investigated the quantity of particulate matter and bioaerosols in the hospital toilet in three distinct scenarios, including neither waste nor flushing, flushing without waste, and fecal matter with flushing a toilet. The bioaerosols concentration was measured 0.15 m, 0.5 m, and 1.0 m from the rims of the toilet, with 5, 10, and 15 min before and after flushing the toilet. The findings suggested that the amount of bioaerosol increased in the toilet space in the case of feces flushing. Additionally, the contrast in the concentrations of bioaerosol was not found at various points in time, indicating that bioaerosols existed in indoor air environments for a prolonged retention time (longer than 30 min after flushing). They summarized that feces and flushing in nosocomial toilets exacerbated the condition towards bioaerosol increase [94]. Besides, Gormley et al. employed two techniques to assess the emission of bioaerosols occurrence. The Aerodynamic Particle Sizer (APS) data indicated that the majority of particles (> 99.5%) were smaller than 5 μm and hence classified as aerosols. During system defect conditions, particles created inside the municipal plumbing system in the form of a flushing toilet cause emissions into the building, including an equivalency by somebody speaking loudly for almost 6 and a half minutes. The result also demonstrated that no particles larger than 11 μm were found in the whole system. The volume of toilet flushing correlated to the population of particles. However, there was a lack of information on the impact of airflow rate on particle count. The number of particles for a 6 L toilet flush was between 3000 and 4000, while for a 1.2 L toilet flush, the total particle was 886–1045. As a result, the decrease in particle size is proportional to the decrease in toilet flush amount [69]. Similarly, Wu et al. introduced a new toilet consisting of a liquid-curtain technique and demonstrated its benefits using computational fluid dynamics. The results showed that the liquid curtain could inhibit upward viral particles in order that a mere 1% of viruses can go up above the toilet, and the water flow can properly transport viruses into the wastewater [95].

Hence, existing scientific research on sewage aerosolization from toilets has demonstrated that flushing a toilet may cause to release of up to 80,000 bioaerosols into the toilet's indoor air and the toilet

environment, floating above the toilet area around one meter (3.2 ft). Therefore, these bioaerosols can easily be floating in air currents due to their low settling velocity, finally depositing on the toilet seat, toilet lid, and the toilet's surface surroundings [90,92,96]. Other authors like Mendes and Lynch observed similar findings. The results of their investigations in public toilets in schools, shops, factories, offices, railway sites, and hospitals showed that bioaerosols were found in the seat of the toilet, washbasin, faucet handle, and floor surrounding toilet. The remarkable point was that some toilet surfaces like the seat of the toilet, the handle of the faucet, washbasin, and interior door knobs were more contaminated than other surfaces [97].

Another issue is that wastewater drainage systems in buildings can serve as a potential reservoir for many bacterial and viral pathogens. Based on recent facts, it seems likely that virus-containing fecal aerosols may be airborne in drainage systems and vents for several hours, regarding being sufficiently small in size. Generally, the hydraulic interactions of toilet wastewater inside vertical building drainage stacks can generate virus-laden aerosol particles after flushing a toilet. The buoyancy (chimney) effect, as well as falling wastewater, can act as a driving force for these virus-contaminated aerosols to move in the drainage stacks and vents transiently. The buoyancy effect is a common phenomenon in high-rise buildings when there is a difference between the air temperature and humidity in the drainage pipes and the indoor air, especially the air in the bathrooms [77,98].

Additionally, the suction flow rate is related to the negative pressure on each flat. A negative pressure situation can result from both toilet or bathroom exhaust fans and a northerly wind that generates a wake flow when there is a bathroom window onto a balcony. In a better word, the negative pressure can cause the generated fecal aerosols in the drainage pipe to suck into bathrooms, and consequently, they can settle on some surfaces; after that, it is possible to spread to other residents or surfaces by touching them [77,98]. For the first time, the hypothesis of a transmission pathway for SARS-CoV-1 through dried-up U-traps, which allowed the virus-laden fecal aerosols to enter households from the plumbing system, was suggested WHO in the 2003 SARS outbreak in Hong Kong. Briefly, dried floor drains have caused the vertical transmission of virus-laden aerosols in the building drainage system in vertically-aligned flats in similar residential tall buildings in southern China during both the SARS-CoV-1 outbreak and the SARS-CoV-2 outbreak. Similar observations were also found in the outbreak of SARS-CoV-1 in Amoy Gardens of Hong Kong [77,80].

4.2. Airborne transmission of SARS-CoV-2 by toilet bioaerosols and the potential risks for occupants

According to recent infectious disease outbreaks worldwide, more scientific attention has been paid to the fecal aerosols containing the virus as a transmission route of infectious diseases in buildings, especially in high-rise residential buildings with dense populations [98]. As mentioned, a wide variety of transmissible pathogens can be carried by human excreta. They enter into sanitation facilities, and building drainage systems can cause a range of potential transport pathways. Research suggests toilets seem to be among the most probable infection and disease transmission spots especially shared toilets in hospitals and workplaces [99].

Since the transmission of SARS-CoV-2 RNA through fecal aerosols was suspected, various studies have investigated whether fecal aerosol may play a role in the transmission of COVID-19 and lead to potential community risks of COVID-19 outbreak. An early report by Ong and al. indicated that the total positive results of surface samples in the patient's bathroom was 60%, which highlighted fecal shedding of viral RNA by patients as a possible way of transmission of the virus [100]. In another recent case report at Fangcang Hospital in China, the highest concentration of SARS-CoV-2 aerosols appeared in a patient mobile toilet room, which was a portable single toilet unit of an approximate one square meter area without ventilation. Therefore, the authors proposed that airborne SARS-CoV-2 could generate during the patient's breath or aerosolized the urine and feces of patients [86]. Results obtained in samples from bathrooms of four isolation rooms in The Second Hospital of Nanjing in China indicated that a tremendous amount of SARS-CoV-2 virus was detected in patients' toilets in the hospital. Thus, the authors suggested the toilet as the most contaminated area of the hospital compared to other parts. In addition, the tested positive surface samples on the ceiling-exhaust grille and the toilet-exhaust louvers recommended the possible existence of fine airborne aerosols in the patient

bathrooms due to patients' exhalation or fecal-derived aerosols during flushing a toilet (Fig. 3) [101].

Moreover, Passos et al. evaluated the presence of SARS-CoV-2 in indoor and outdoor spaces. They collected samples from different wards of two hospitals including, especially patient mobile toilet rooms. The results showed that 1 sample of 62 samples was positive [102]. Similarly, it has been assessed the SARS-CoV-2 stability in the quarantine hotel environment. It was indicated the contamination rates of hand sinks (12.82%), toilet seats and flushes (7.89%), and floor drains (5.41%) [103]. In another study, Cheng et al. believe that the airborne path is not the most common way for SARS-CoV-2 to spread. They examined the air and surface sample of an isolated room that the patient was there. All air samples for SARS-CoV-2 were negative, but SARS-CoV-2 RNA was found in contaminated environments such as patients' mobile phones (6 of 77, 7.8%) and toilet door handles (4 of 76, 5.3%) with a median concentration of 9.2×10^2 copies mL^{-1} (range, 1.1×10^2 to 9.4×10^4 copies mL^{-1}) [104]. Conversely, the results in further research on SARS-CoV-2 air contamination evaluation in medical settings showed that the positivity rating was 5 of 21 air samples (23.8%) with concentrations of 9.7×10^3 copies m^{-3} (5.1×10^3 to 14.3×10^3 copies m^{-3}) in the air of toilets or bathrooms [105]. It should be mentioned that the SARS-CoV-2 contamination in the six bathrooms of the non-ICU isolation ward in West China Hospital before routine cleaning was not remarkable and high, while in several studies, the contaminated areas in the toilet have been seen in dedicated COVID-19 hospitals [106]. The authors mentioned flushing a toilet daily with 2000 mg/L chlorine solutions as a possible reason [107]. Evidence from other studies, as tabulated in Table S3 (Supplementary material), indicated that the presence of the SARS-CoV-2 in the toilet bowl and flush button environments of hospital and quarantine room and investigation on latrines and flushing toilets in detecting SARS-CoV-2.

Döhla et al. analyzed siphon and toilet wastewater samples in 21 private households where at least one individual was infected with

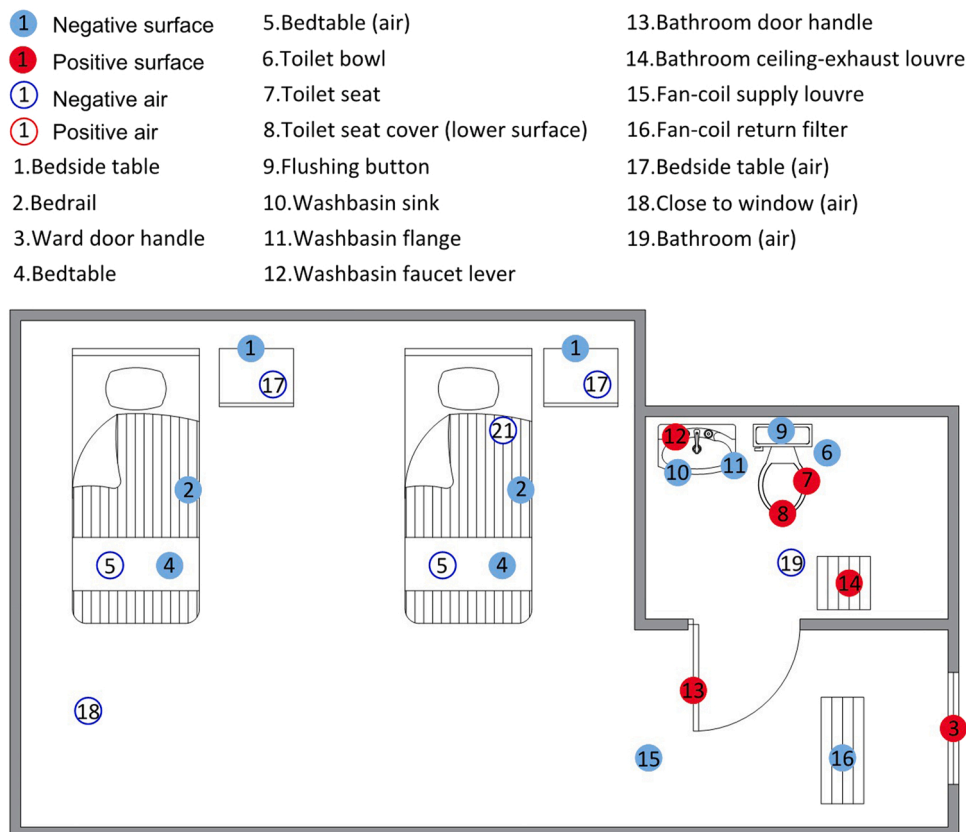


Fig. 3. Sampling site in a quarantine room [101].

SARS-CoV-2 RNA under quarantine conditions in March 2020 in Germany. Their result (Table 1) reported 19.23, 18.75, and 8.70% of the detected positive wastewater samples in the washbasin siphons, the shower siphons, and toilets, respectively (Table 1). They indicated that a high amount of viral load in the wastewater of sanitation units could be considered a possible source of infection with SARS-CoV-2. Also, they could support the hypothesis of toilet flushing-generated aerosols of SARS-CoV-2 as a potential transmission route [108].

To assess fecal aerosols' possibility of spreading COVID-19 infection in a high-rise building, Kang and colleagues assessed nine members of three families who were confirmed with COVID-19. The infected people lived in vertically aligned -02 flats (No. 1502, 2502, and 2702) connected by master bathroom drainage stacks and vents in a high-rise building (block X) in Guangzhou, China in a period of social distancing (Fig. 4b). It should be mentioned that block X was a 29-floor residential high-rise with 3-unit apartments (flats 01, 02, and 03) on each floor except the 29th floor (Fig. 4a).

To investigate the virus transmission via fecal aerosols, the authors collected data on environmental samples from different locations within the building, especially the master and guest bathrooms (Table 2). The authors revealed that the master bathroom bathtubs of most surveyed families living in 11 of 16 -02 flats (containing the infected residents of apartments 2502 and 2702) were expected their water seals to be dried out because of families' usage habits. In the absence of evidence about dried-out water seals in 2502 and 2702 due to instant disinfection, it was impossible to determine. However, the detection results of tracer gas concentrations, which were released into the drainage pipe through the toilet of apartment 1502, displayed the possibility of bioaerosols entering the environment of the bathroom via drainage stacks in mentioned flats. The authors reported that floor and bathtub drains could act as a transport path for fecal bioaerosols between the flats if the drain water seals dry up due to nonuse for an extended period.

Moreover, the authors suggested that fecal bioaerosols could leak back into the same bathroom or other places linked to the drainage system due to the dried-out floor or bathtub drain. They cited the positive surface samples in the bathrooms of flats 1602 and 1502 (Table 2) as the reason for their concept. In addition, they described the phenomenon of how the same suck-in might happen in the bathrooms located in the -02 flats. They proposed that whenever another resident in the upper flats was in their bathrooms while the toilet in the index patient's bathroom was used and flushed, they might inhale some bioaerosols that were sucked into the master bathrooms from drainage pipes. Furthermore, according to the indoor location of the stack and vent in the master bathroom, they demonstrate the role of a buoyancy (chimney) effect for the bioaerosols movement temporary within the drainage stacks and vents. Whenever there was a minimal difference in air temperature and humidity of the drainage pipes and the bathrooms, a considerable stack effect because of the "chimneys" would be the result. They also mentioned the effect of the negative pressure due to using an exhaust fan or by a northerly wind on the suction volume flow of each apartment. They reported the positive sample in the bathrooms of uninhabited flat 1602 (Table 2) to support the view of generating negative pressure by wind because of turning off its exhaust fan at that time.

Although a positive sample detected from inside the U-trap of the master bathroom washbasin was considered as a reason for generating fecal aerosols in the drainage pipes, the authors had no direct evidence for the existence of fecal-derived aerosols in the drainage pipe system. The infection risks of residents living in -02, and -01 and -03 flats of block X were also evaluated 3/60 and 0/136, respectively. Therefore, obtained results recommended that the greater infection risk posed to the individuals in -02 flats compared with those in -01 and -03 [77].

Two similar cases were also observed in Hong Kong. Those results seem to indirectly confirm the possibility of vertically aligned flats to facilitate the spread of SARS-CoV-2 within, or even between, apartments because of the common vertical drainage pipes [109,110].

The ability of organisms to persist and survive for a long period on a surface highlights the importance of infection risk by a deposited pathogen. Several studies have been conducted on evaluating the stability of viral pathogens, especially the SARS-CoV-2, on various surfaces to determine their decay rates. According to recent investigations, SARS-CoV-2 can survive on metals, glass, and plastic from hours to days at room temperature. For example, Doremalen et al. found that SARS-CoV-2 could be alive on different surfaces for 3 days; the results suggest that the SARS-CoV-2 seems to be more stable on plastic, stainless steel, which was detected viable after 2–3 days [111]. Another study also estimated SARS-CoV-2 half-lives on indoor surfaces, including cloth, styrofoam, cardboard, concrete, rubber, glass, stainless steel, galvanized, and steel under three seasonal conditions, which are exhibited in Table 3.

The exposure infection risk of viral SARS-CoV-2 aerosols created by either toilet flushing or building drainage systems has been less investigated. At the time of this review, only Shi et al. estimated the exposure risk of infection by quantitative microbial risk assessment (QMRA) in two scenarios (Table 4). The results showed that the median risks of illness with COVID-19 per exposure for a single day were estimated 1.11×10^{-10} and 3.52×10^{-11} for two scenarios of flushing a toilet and faulty drainage, respectively. Moreover, the estimated exposure of the worst-case scenario, highly polluted aerosols after flushing toilets in the hospital bathroom, was reported 1.9×10^{-6} per person per event [113].

As presented, aerosolized viruses formed during toilet flushing processes can contaminate the toilet's indoor air and the toilet environment, especially the nearby surfaces such as toilet seats, lids, and surrounding floors. Thus, toilet plume droplets may contribute to the spread of infectious diseases, especially the COVID-19 coronavirus. As a result, toilet plumes may pose a risk for public health during pandemics [90]. Although the role of airborne virus transmission of SARS-CoV-2 by toilet flushing is not clearly described due to difficulties associated with identifying this route of transmission for any case of outbreaks, toilets must be pondered as a possible pathway of the spreading virus through indoor air and viral surface contamination [114]. So, further research is needed to supply evidence of airborne transmission risk of SARS-CoV-2 because of toilet flushing and contact transmission risk due to contaminated surfaces by flush droplets, particularly extremely contaminated surfaces within toilet rooms.

Table 1

The analysis of viral SARS-CoV-2 RNA in wastewater of households during quarantine conditions.

Ref.	Location	Reference site	Sampling period	Reference wastewater subtypes	Reference sample collection condition	Reference sample		Reference comment
						(Positive/total)	%	
[108]	Germany	Wastewater samples of the shared sanitary facilities in 21 private households COVID-19 infected people	in March 2020	Washbasin siphons	Before routine Cleaning	5/26	19.23%	The highest
				Shower siphons		3/16	18.75%	higher
				Toilet		2/23	8.70%	The lowest
				Other		0/1		

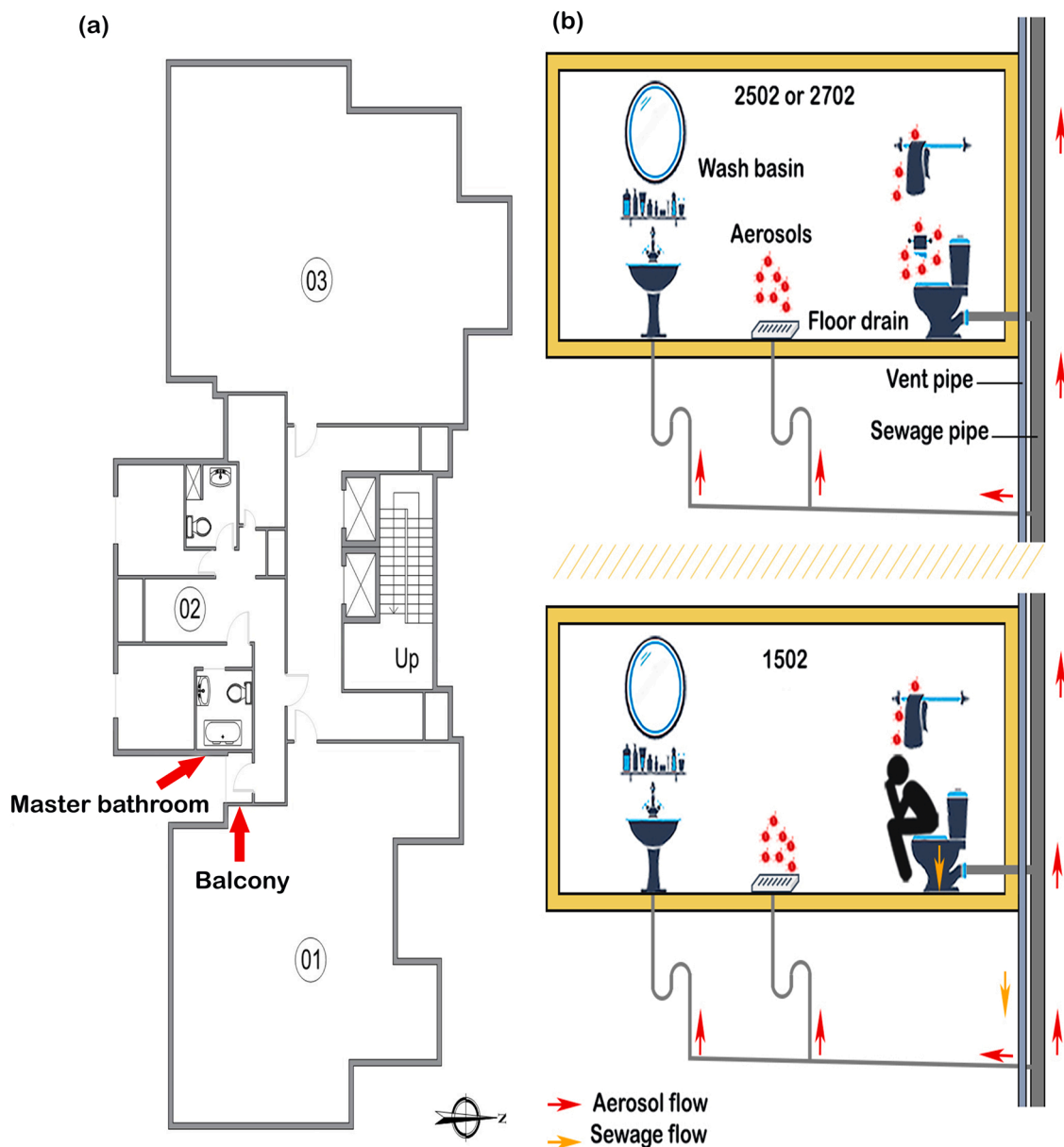


Fig. 4. Transmission route of SARS-CoV-2 in a high-rise building in China, a) plan, b) section floor [77].

4.3. The health aspect of aerosol

As mentioned, one of the most important elements of particulate matter (PM), which plays a crucial role in human health, is bioaerosol. It provokes cancer, severe toxic effects, diabetes, neurological diseases, hypertension, cardiovascular diseases, infectious diseases, and allergies. Most bacterial diseases like Tuberculosis, Anthrax, and Legionellosis are caused by long-term or even short-term exposure to bioaerosols [115, 116]. It can be argued that some bacteria, viruses, and fungi in the form of aerosols have prolonged stability in the air. It has been reported that bacteria with the size of ($PM_{10-2.5}$) remain in the air. The aerosols in which humans breathe into the respiratory tract deposit in different locations of the respiratory system with various precipitation rates (Fig. 5) [116]. For this reason, it gives significant indications for analyzing the possible health consequences of aerosols.

5. Aerosolized SARS-CoV-2 within wastewater treatment plants

5.1. Factors affecting bioaerosols emission from wastewater treatment plants

Wastewater treatment plants are identified as one of the significant sources of emitting microbial bioaerosols, which have attracted extensive attention because of their potential health risks to sewer workers and the surrounding people [71,74,76,117–122]. In recent decades, many studies have focused on investigating bioaerosols in WWTPs to determine association with bioaerosols emission and physical-chemical parameters [123–125].

The amount of the concentration of the pathogen in bioaerosols, releasing into the environment of wastewater treatment plants, relies on various factors including, environmental conditions (relative humidity (RH), temperature, solar radiation (SR), wind speed, and season), time of the day, distance from the source of aerosol, the equipment utilized in the mechanical phase of a WWTP, daily inflow at the WWTP, and the survival of microbial bioaerosols in the open air [75,126–128].

Table 2

Summary SARS-CoV-2 detections in the air or surface of the bathrooms in Block X.

Ref.	Location	Reference site	Number		Sampling date	Reference area	Reference samples (positive/total)	
			Infected patients	Others			Air	Surface
[77]	China	Vertically aligned flats in a high-rise apartment building (Block X) in Guangzhou		2802	13 Feb 2020	Frequently touched surfaces, air supply inlets, drains	0	0/28
						Floor drain air in master and guest bathrooms	0/2	
			2702		14 Feb 2020	Bathroom, various surfaces		0/9
				2602	14 Feb 2020	Master bathroom, various surfaces		0/14
						Guest bathroom, various surfaces		0/7
			2502		14 Feb 2020	Master bathroom, bathtub drain, exhaust fan		0/2
				2302	14 Feb 2020	Master bathroom, various surfaces		0/8
						Guest bathroom, various surfaces		0/6
				1802	14 Feb 2020	Bathroom, various surfaces		0/7
				1702	13 Feb 2020	Frequently touched surfaces, air supply inlets, drains		0/20
						Floor drain air in master and guest bathrooms	0/1	
				1602	13 Feb 2020	Frequently touched surfaces, air supply inlets, drains, Floor drain air in master and guest bathrooms	0/2	1/15
						Light switch in the guest bathroom		0/1
			1502		12 Feb 2020	Frequently touched surfaces in master bathroom		3/4
						Floor drain and mop in the master bathroom		0/2
[112]	China	Vertically aligned flats in a high-rise apartment building (Block X) in Guangzhou				Door handle, washbasin, & tumbler in the guest bathroom		0/2
					19 Feb 2020	Master bathroom, washbasin U-trap inner surface		1/1
				1402	13 Feb 2020	Frequently touched surfaces, air supply inlets, drains		0/25
						Floor drain air in master and guest bathrooms	0/2	

Table 3

SARS-CoV-2 half-life on various surfaces in indoor and three different seasons [112].

	21 °C/60% RH (indoor condition) half-time (h)	25 °C/70% RH (summer season) half-time (h)	13 °C/66% RH (spring/fall season) half-time (h)	5 °C/75% RH (winter season) half-time (h)
Cloth	3.5	2.99	19.94	47.94
Styrofoam	9.62	4.75	24.67	112.91
Cardboard	12.86	5.03	26.93	121.78
Concrete	7.96	2.54	17.11	80.99
Rubber	11.33	5.03	28.27	115.74
Glass	9.6	5.58	27.34	92.03
Stainless steel	7.75	3.41	23.46	70.06
Galvanized steel	6.93	4.19	24.22	67.21
Tyvek	9.36	4.57	31.82	90.59
Polypropylene	9.02	4.51	28.75	75.54
Nitrile gloves	11.56	4.42	22.94	85.71
N95 mask	9.01	4.4	27.77	106.37

5.1.1. Influencing the natural conditions derived from a WWTP

Meteorological parameters are considered the main factors are effecting on bioaerosol emission from WWTPs. Based on the collective data, bioaerosol concentration distributions in a year are variable, considering the ambient air temperature and region. Studies on the concentration levels of bioaerosol generated at WWTPs have indicated that the highest concentrations occurred in the summer due to the suitable temperature, relative humidity, and wind speed [71,74,125,

128,129].

Overall, bioaerosols structures are positively correlated with relative humidity due to pathogens' survival, influenced by relative humidity levels in the air [123]. Results obtained by Jones and Harrison indicated that an RH of 70–80% and a temperature of 12–15 °C led to the survival and proliferation of bioaerosols in the air [130]. In another study by Wang and et al., the RH range and the ideal temperature were 37.8–57.4% and 27.6–40.1 °C in the summer, respectively, to have maximum numbers of pathogens in the air. Also, Wang's results agreed with Han's research report, which suggested that RH of 40–60% and temperature of 20–37 °C led to the survival and proliferation of bioaerosols in the air [123,125].

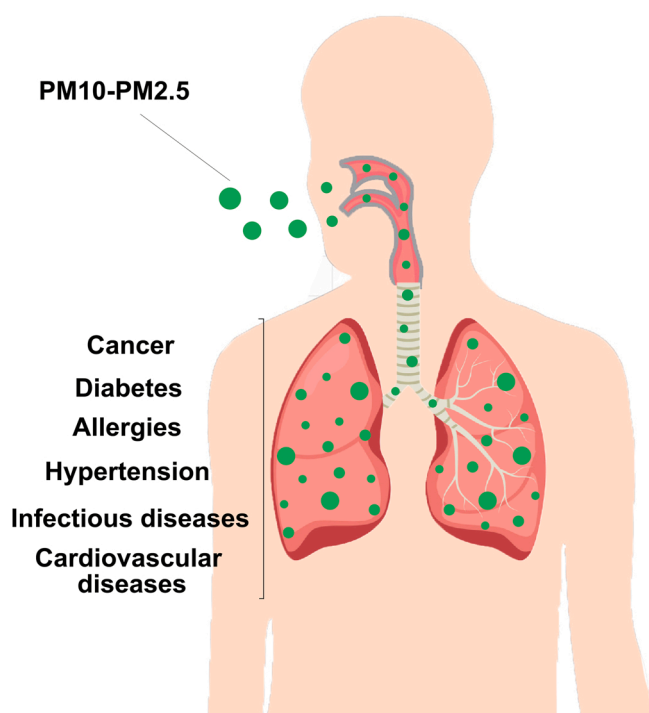
It seems that the survival of viable airborne pathogens is greatly negatively affected by solar illumination because pathogens can be inactivated by ultraviolet irradiation. As a result, this causes the death of pathogens in bioaerosols. It was also observed that high temperatures could control the number of airborne pathogens at WWTPs [118].

The impact of wind speed on airborne pathogens has not been understood, and more studies are necessary [131]. Han and et al. found that high wind speed could result in more remarkable pathogens in the near-surface layer of wastewater, which caused high suspensions of pathogens in the air. In contrast, several studies have reported a negative impact of wind speed on microbial aerosol loading, and as a consequence, a decrease in the bioaerosols level due to dispersion and dilution by the wind. In addition, wind can decrease pathogen aerosolization's ability to survive [76,123,125,126]. In particular, Fracchia et al. suggested the low amount of airborne pathogen contamination was detected at downwind sites as a result of the bioaerosol dilution according to the distance from the sources [124].

Table 4

Summary characteristics of the recent exposure risk assessment (QMRA) of toilet aerosols and indoor drainage system for SARS-CoV-2.

Ref.	Reference scenario	Condition	Considered factors of QMRA	Reference exposure scenario	Reference level of risk ^a
[113]	Toilet flushing	<ul style="list-style-type: none"> – A bathroom was shared with other roommates – The only one of them was a COVID-19 patient on self-isolation in his/her private room 	<ul style="list-style-type: none"> – Aerosols concentration in air after flushing a toilet at various heights above the toilet – Half-life of SARS-CoV-2 in the toilet-generated aerosols – Interval between the prior toilet flushing of patient's feces and the healthy suitemate using the toilet room – Deposition efficiency of aerosols in the respiratory tract – Time spent in restroom after one toilet flushing – Flow rate during human breathing 	Median	1.11×10^{-10}
	Faulty drain	<ul style="list-style-type: none"> – The hospital toilet room – A patient with COVID-19 isolated his/herself at their flat – A neighbor in a flat one floor below the index patient with shared main sewer soil vent pipe and dried-out water seals 	<ul style="list-style-type: none"> – Median diameter aerosol sizes – Concentration of aerosols in drainage riser – The size of toilet room – Dispersion rate of aerosol – Inhalation of polluted aerosols by the downstairs neighbor during using the toilet by the upstairs neighbor 	Worst Median	1.9×10^{-6} 3.52×10^{-11}

^a illness risk per exposure event (in per person per event).**Fig. 5.** Deposition of aerosols in different locations of the respiratory tract.

5.1.2. Influencing the treatment sections on bioaerosols derived from a WWTP

Different operational activities at WWTP can generate bioaerosols, especially in which parts include moving mechanisms and aeration. Recent studies conducted to investigate emissions from various stages of the wastewater treatment plants have been described that bioaerosol characteristics and the amounts of emission vary significantly due to the stage of the process and the technique utilized in the process of wastewater treatment. In most studies, the high level of bioaerosols was observed during pretreatment (such as bar screens, pump stations, and grit chambers), biological treatment (such as aeration tanks), and sludge treatment operations at a WWTP [71,74,118,125,132].

According to published studies, high bioaerosol emission has been found in the air of mechanical sewage treatment devices such as grit

chambers and bar screens. A research group reported that the highest airborne pathogens level was noted at the aerated grit chamber in Greece. Similar observations were made in another study in the covered grit chamber at the WWTP in Austria [119,124]. In fact, the continuous flow of raw wastewater into the grit chamber can cause to generate a great number of bioaerosol droplets that the wind can scatter. Besides, screw conveyors, which can carry sludge or solid matter from the grit chamber to a storage container, are another one to generate and emit bioaerosols in the air in the WWTP's area [133]. In bar screens, which are required routine cleaning manually or mechanically, the water surface is regularly disturbed by mechanical devices such as the drive chains or by hand cleaned racks that result in the release of bioaerosols in the air surroundings of the WWTPs [76,118].

Similarly, the authors stated that biological treatment, based on the aeration of wastewater, either by mechanical agitation or air bubbling, was determined as one of the main bioaerosol emission sources at a WWTP. In general, aeration, which provides oxidative processes in the aeration tanks, is achieved with diffused aeration located at the bioreactor bottom or mechanical surface aeration. Therefore, bioaerosol emission from aeration basins is associated with types of aeration and mixing of liquids by mechanical agitation [71–73,124,132]. In better words, bioaerosols release from aeration basins because of the intense mixing and turbulence; it means that a large number of pathogens can be entered into ambient air during aeration as a consequence of splashing and bubble bursting. The study of Sánchez-Monedero et al. showed that the bioaerosol levels generated were influenced by the type of aeration utilized in the biological process. Consequently, this may result in different exposure risk levels of site workers. In addition, the results of similar studies confirmed that mechanical aeration – especially horizontal rotors and surface turbine aerators – has generated higher levels of bioaerosols than bubble diffused air systems. However, a tremendous amount of respirable bioaerosols is generally generated by bubble aeration [72,76,118,132].

Likewise, airborne pathogens emitted from different types of sludge treatment was observed in sludge storage site and various units of sludge treatment such as the sludge dewatering chamber, thickening basin, and the sludge centrifugation process [71,125].

Obtained results of different WWTP stages also revealed that the highest amount of airborne pathogens was detected in the pretreatment process compared to other parts. This might result from the worse microbiological quality of wastewater in the first stages since the wastewater is still wholly untreated. The decrease in pathogen contamination levels might be due to the sewage become more treated

[124,126]. Thus, microbial bioaerosol emission levels will begin to decline from pretreatment to primary, secondary slowly, and the advanced treatment methods of wastewater [92]. Generally, virus removal in secondary treatment technologies is approximately 90% from raw sewage. However, conventional wastewater treatment processes cannot remove all viruses from sewage [64].

For example, Pascual et al. reported decreased airborne pathogen concentration at aeration tanks, where wastewater is further treated and purified beyond the primary treatment stage. The authors also reported similar results for the sludge processors due to becoming inactive a significant percentage of pathogens [126].

5.2. Fecal bioaerosol transmission of SARS-CoV-2 and potential risks of infection for wastewater operators/workers

Infectious agents, such as respiratory viruses, could be transported in aerosols formed during processes of wastewater treatment, and thus, the aerosolization of the virus-infected droplets is likely to spread viral infections to workers in wastewater treatment plants [54,134]. As has been illustrated by Khuder et al. and other studies, there is a critical correlation between exposure to wastewater bioaerosols and the occurrence of infectious diseases at WWTPs. In the better word, site workers may be exposed to fecal bioaerosols containing various infectious agents, including viruses, through inhalation or by the hand-to-mouth route. Hence, fecal aerosols could be considered a possible transmission route and pose a risk to workers at WWTPs and groups living around them. There is much evidence of this fecal-aerosol transmission pathway for various enteric viruses and bacteria. In other words, exposure to fecal bioaerosols, due to a potential risk of spreading the virus among the sewer workers, has been regarded. It becomes crucial when the infective pathogens can be easily transmitted from the exposed workers to their family members and other friends and relatives [118,135]. Literature data demonstrated that wastewater operators - depending on kinds of facilities, carried out work, and climate conditions - may be exposed to the unpredictable amount of fecal bioaerosols [117,124,136–138]. In particular, the inflow chambers and treating processes that produce splashing, bubbling, and spraying have proved to be of greatest potential risk for site workers. Thus, during wastewater treatment processes, a significant exposure risk for sewage workers is posed by the screen room, sludge treatment processes, and the biological reaction basin of a WWTP. However, it should be mentioned that although fecal bioaerosol concentrations at the office building are significantly lower than other parts, it might still be polluted by microbial species transported by the workers' clothing and shoes from other stages of the WWTP [119].

Importantly, in all studied wastewater treatment plants, the technology and the type of the treatment process seem likely to lead to differences in emission levels and exposure to bioaerosols. For example, Sanchez-Monedero et al. obtained results that indicated that concentrations of emitted bioaerosols in the biological process are greatly influenced by the type of aeration applied. As a result, the risk levels of plant workers from exposure depend on them [132]. In another study, Yang et al. found that the outdoor wastewater treatment processes such as aeration parts and a sludge dewatering room generated various microbial aerosols that may cause high exposure risks microbial aerosols at WWTPs [122].

In view of processes, they differ mainly in being performed outdoors or indoor. In indoor sewage treatments where the main stages of the processes occur in a closed area, the concentrations of airborne pathogens in the ambient air are higher due to inadequate ventilation and a low rate of die-off from the absence of sunlight. Moreover, pathogens could survive better in indoor WWTPs. Therefore, the working areas of indoor wastewater treatment plants pose a higher risk of exposure to bioaerosols [118]. In fact, there is wide variation in the number of bioaerosols across different studies because of differences in methods of wastewater treatment and sludge, meteorological conditions as well as

in sampling and statistical methods [137,139,140]; consequently, no uniform international standard have been set up for estimating indoor and outdoor airborne pathogen concentrations [119].

Furthermore, in recent years, there is a great concern about the particle size of bioaerosols, which influences the exposure risk of respiratory diseases to the staff at a WWTP and surrounding residents [125]. Previous studies have reported that most bioaerosol particles in the air of a WWTP are in the respirable size range, diameter below 4.7 μm , which can raise the risk of penetrating deep into the lungs of exposed site workers. It has also been reported that the size distribution of bioaerosols significantly differs in various seasons. Of note, these small bioaerosols can be transported by the wind to distances between a few hundred meters to several kilometers. Therefore, they can pose a serious biohazard threat not only to sewage workers but also to neighborhood residents. Another issue of great concern is linked to the high ability of viruses to infect humans. This means that the minimum infective dose of airborne viruses is enough to cause an infection [71,73,75].

In the case of SARS-CoV-2, knowledge on SARS-CoV-2 aerosolization within WWTPs is limited, even though recent initial studies have confirmed the presence of SARS-CoV-2 RNA in wastewater influents of a WWTP in the Netherlands, France, Germany, Spain, Italy, Australia, the United States, and Turkey (Table 5).

In particular, the WHO has published brief literature data, which are in agreement with these reports. Despite these reports, there is a lack of enough information about the fate of SARS-CoV-2 during the different wastewater treatment stages, and consequently, few reports investigated aerosolization of SARS-CoV-2 during different stages of wastewater collection and treatment [3]. Thus, based on previous evidence, it can be concluded that the risks of aerosolization of SARS-CoV-2 can be extremely significant during pumping wastewater, discharge from sewerage networks, and in uncovered aerobic treatment units. This is why raising concerns about the exposure to SARS-CoV-2 emerged from the fecal-derived aerosols due to the considerable SARS-CoV-2 RNA load to WWTPs [54,152]. Therefore, in few recent studies (Table 6), quantitative microbial risk assessment (QMRA) has been used to evaluate potential health risks of SARS-CoV-2 in aerosolized wastewater for wastewater workers linked to exposure. In the early study of conducting a QMRA of SARS-CoV-2 in two WWTPs in Brazil, Zaneti et al. estimated the exposure risk of SARS-CoV-2 infection among workers with presuming three various scenarios of COVID-19 pandemic and two consideration items of QMRA. The results revealed that the calculated infection risk for the extreme, aggressive, and moderate scenarios were up to 1.3×10^{-2} , 2.6×10^{-3} , and 1.2×10^{-4} , respectively. Moreover, the tolerable risk of infection per person per year (pppy) for SARS-CoV-2 was reported above 5.5×10^{-4} , which strengthened the worry about a possible transmission route of SARS-CoV-2 via sewerage systems [177]. However, the later study of QMRA by Dada and Gyawali in New Zealand was not confirmed the view of the accidental risk exposure of WWTPs workers to SARS-CoV-2 in sewage by inhalation or ingestion of the ambient air surrounding treatment plants. The authors assessed the risk of illness to operators in three different scenarios and six consideration factors. The authors estimated the negligible exposure risk for the site workers (0.036, 0.32, and 3.21 illness cases per 1000 exposed workers at WWTP, respectively, for low grade, moderate and aggressive scenarios) [178]. The later study of QMRA by Gholipour et al. found that the range of infection risks of SARS-CoV-2 for WWTP operators was from 1.1×10^{-2} to 2.3×10^{-2} pppy by analyzing the viral SARS-CoV-2 RNA presence in raw sewage and samples of air from surroundings of the WWTPs. The obtained results by the authors indicated that The estimated level of the annual risk of infection for treatment plant operators was greater than the suggested level by WHO (10^{-3} pppy). Therefore, according to a few current investigations in the risk exposure assessment, further research is quite necessary [179].

Table 5

Detection of SARS-CoV-2 in untreated wastewater in the globe.

Ref.	Region	Water type	Method analysis	Number concentration in influent (copies/lit)
[141]	Detroit, USA	Untreated wastewater	RT-qPCR	1.24×10^4 – 4.33×10^5
[142]	Gandhinagar, India	Untreated wastewater	RT-qPCR	August: 897.5 September: 924.5
[143]	Murcia, Spain	Untreated wastewater	RT-PCR	1.48×10^5 – 3.9×10^5
[144]	Virginia, USA	Untreated wastewater	RT-ddPCR	10^1 – 10^4
[33]	Australia	Untreated wastewater	RT-qPCR	19–120
[145]	Yamanashi Prefecture, Japan	Untreated wastewater	RT-qPCR	Not detected
[36]	Louisiana, USA	Untreated wastewater	RT-qPCR	3.5×10^3 – 7.5×10^3
[18]	Milan, Italy	Untreated wastewater	RT-PCR	No data available
[24]	Ahmedabad, India	Untreated wastewater	RT-PCR	5.6×10^1 – 3.5×10^2
[146,147]	Italy	Untreated wastewater	RT-PCR, RT-qPCR	Below the identification threshold to 5.6×10^4
[26]	Japan	Untreated wastewater	RT-qPCR	1.2×10^4 – 4.4×10^4
[148]	Belgrade, Serbia	Untreated wastewater	RT-qPCR	5.97×10^3 – 1.32×10^4
[149]	Valencia, Spain	Untreated wastewater	RT-qPCR	1.66×10^5 – 9.77×10^5
[28]	Montana, USA	Untreated wastewater	RT-qPCR	$> 10^4$
[150]	Canada	Untreated wastewater	RT-ddPCR, RT-qPCR	PGS (RT-ddPCR): 1.24×10^6 – 1.42×10^6 PGS (RT-qPCR): 1.42×10^6 – 1.93×10^6 PCS (RT-qPCR): 1.10×10^6 – 1.51×10^6 PCS (RT-ddPCR): 2.74×10^5 – 3.93×10^5
[17]	Rio de Janeiro, Brazil	Untreated wastewater	RT-qPCR	Cycle threshold numbers are reported
[151]	Paris, France	Untreated wastewater	RT-qPCR	5×10^4 – 3×10^6
[152]	Israel	Untreated wastewater	RT-qPCR	Cycle threshold numbers are reported
[19]	Istanbul, Turkey	Untreated wastewater	RT-PCR	Primary and activated sludge: 1.17×10^4 – 4.02×10^4
[153]	USA	Untreated wastewater	RT-qPCR	Primary sludge: 1.7×10^6 – 4.6×10^8
[154]	New York, USA	Untreated wastewater	RT-qPCR	Below the identification threshold to 1.12×10^5
[155]	Ourense, Spain	Untreated wastewater	RT-qPCR	7.5×10^3 – 4×10^4
[156]	Pakistan	Untreated wastewater	RT-qPCR	Quantification cycle numbers are reported
[157]	Barcelona, Spain	Untreated wastewater	RT-qPCR	$< 10^5$
[158]	Santa Catalina, Brazil	Untreated wastewater	RT-qPCR	2.95×10^5 – 5.01×10^6
[159]	Santiago, Chile	Untreated wastewater	RT-qPCR	Not detected– 4.81×10^6
[160]	Jeddah, Saudi Arabia	Untreated wastewater	RT-qPCR	Septic tank: 1.74×10^2 – 1.33×10^3 Hospital: 1.4×10^4
[161]	San Francisco, USA	Untreated wastewater	RT-qPCR	1.07×10^3 – 1.02×10^6
[162]	USA	Untreated wastewater	RT-qPCR	Nine states higher than 100
[163]	Florida, USA	Untreated wastewater	RT-ddPCR	9.47×10^5 – 2.19×10^6
[164]	India	Untreated wastewater	RT-qPCR	Vadodara: 3078 Ahmedabad: 2968 Gandhinagar: 354
[27]	Netherland	Untreated wastewater	RT-qPCR	2.6×10^3 – 2.2×10^6
[165]	United Arab Emirates	Untreated wastewater	RT-qPCR	2.86×10^2 – 2.90×10^4
[166]	Australia	Untreated wastewater	RT-ddPCR, RT-qPCR	Below the identification threshold to 8.80×10^3
[167]	Tehran, Iran	Untreated wastewater	RT-qPCR	7.18×10^2 – 1.09×10^4
[168]	India	Untreated wastewater	RT-PCR	No available quantitative data
[11]	Virginia, USA	Untreated wastewater	RT-qPCR	Composite samples: 2.5×10^2 – 1.1×10^4 Grab samples: 5.8×10^3 – 1.38×10^4 Not detected– 3.55×10^7
[169]	Lahore, Pakistan	Untreated wastewater	RT-qPCR	Cycle threshold numbers are reported
[170]	Hyderabad, India	Untreated wastewater	RT-PCR	Municipal: 7.4×10^3 Hospital: 3.8×10^3 – 9.3×10^3
[171]	Wuhan, China	Untreated wastewater	RT-qPCR	Cycle threshold numbers are reported
[172]	Canada	Untreated wastewater	MPAD	Cycle threshold numbers are reported
[173]	Rajasthan, India	Untreated wastewater	RT-qPCR	Cycle threshold numbers are reported
[174]	Frankfurt, Germany	Untreated wastewater	RT-qPCR	WWTP, Niederrad: 4×10^{11} – 1×10^{15} copies/day WWTP, Sindlingen: 1×10^{11} – 2×10^{14} copies/day
[175]	Hangzhou, China	Untreated wastewater	RT-qPCR	Septic tank influent: 5.89×10^5
[176]	Germany	Untreated wastewater	RT-qPCR	Solid stage: 2.5×10^4 Aqueous stage: 3×10^3 – 2×10^4

6. The fate of SARS-CoV-2 RNA in treated wastewater

In the 21st century, the role of water reclamation in order to protect water resources and the environment is not negligible. Generally, water reclamation is the reuse of wastewater after the suitable treatment process for the purposes such as the irrigation of agricultural land or replenishing surface water and groundwater. It is noteworthy that wastewater reuse is highly beneficial for the environment, as it can provide opportunities for communities to have the ability to access enough water for different purposes without requiring to utilize plenty of potable water [180]. Therefore, regarding the increasing need for

wastewater reuse globally, it is critical to consider the role and potential of reclaimed wastewater in the spread of infections, especially in recent outbreaks [3].

As noted above, conventional wastewater treatment plants are not specifically designed to remove viruses in full-scale. On the whole, typical secondary wastewater treatment processes are usually able to achieve an average of 90% removal for viruses [54]. For instance, Prevost and colleagues observed that the viral load of treated wastewater effluents was decreased by 100 times compared to raw wastewater [151, 181]. Even though the level of removals seems widely changeable (ranging from inconsiderable to a 99% reduction), some supplementary

Table 6

Summary characteristics of the recent case studies for the exposure risk assessment (QMRA) of WWTPs workers to SARS-CoV-2.

No	Reference	Location	Site	Treatment process	Considered factors of QMRA	Reference exposure scenario	Reference level of risk	Reference annual infection risk (pppy) ^a
1	[177]	Porto Alegre, Brazil	SJN-WWTP ^b	Manual (coarse) & Automatic (fine) screening, Grit removal, UASB reactor, Aerobic stage Sludge generated → UASB reactor & dehydrated by Centrifuges	– Viral loads associated with the number of infected individuals – Assuming 1 mL of sewage including SARS-CoV-2 as the volume ingested by WWTP operators who exposed to the pre-aeration stage	Extreme ^c Aggressive ^d	1.1×10^{-2j} 2.2×10^{-3j}	5.5×10^{-4}
			S-WWTP ^f	Manual (coarse) & Automatic (fine) screening, Grit removal, Activated sludge, Secondary settling Sludge generated → Activated sludge & dehydrated by Centrifuges		Moderate ^e Extreme ^c	1.0×10^{-4j} 1.3×10^{-2j}	
						Aggressive ^d	2.6×10^{-3j}	
						Moderate ^e Aggressive ^g	1.2×10^{-4j} 3.21^h	
2	[178]	New Zealand	more than 300 publicly owned WWTPs	Wastewater stabilization ponds → wholly or in cooperation with	– The population proportion of infected people			
				Activated sludge, Aeration lagoons, Wetlands, Sand filters, Membrane filtration, & Ultraviolet based disinfection	– SARS-CoV-2 level in effluent & influent WWTPs – Inhaled aerosol volume – Humidity and Temperature – Exposure assessment – Doses of exposure	Moderate ^h Low-grade ⁱ	0.32^a 0.036^a	
3	[179]	Iran	WWTP A ^j	Pumping station, Aerated grit chamber, Aeration tank (Activated Sludge)	– Population infection percentages			$1.1 \times 10^{-2} - 2.3 \times 10^{-2}$
			WWTP B ^k		– Levels of viral RNA in wastewater and aerosolized wastewater samples – Exposure assessment – Doses of exposure			

^a Per person per year.^b São João Navegantes wastewater treatment plant.^c Based upon New York City's population (USA) in April 2020 and the total of active COVID-19 cases → the result of 22% of population.^d Based upon Madrid's population (Spain) in April 2020 and the total of active COVID-19 cases → the result of 4.6% of population.^e Based upon Porto Alegre's population (Brazil) in April 2020 and the total of active COVID-19 cases → the result of 0.2% of population.^f Serraria wastewater treatment plant.^g 3% of infected individuals presumed to be responsible for SARS-CoV-2 stool shedding in wastewater.^h 0.3% of infected individuals presumed to be responsible for SARS-CoV-2 stool shedding in wastewater.ⁱ 0.03% of infected individuals presumed to be responsible for SARS-CoV-2 stool shedding in wastewater.^j Illness case per exposed WWTP operators.^k South of Isfahan (Iran).^l North of Isfahan (Iran).

wastewater treatment units (such as filtration and disinfection processes) are most commonly added to the current secondary treatment system to decrease the amount of virus in order to provide acceptable levels of effluent quality for discharging into the environment [180, 182].

The findings of previous studies on the presence of enteric viruses of humans in raw and treated wastewater indicated that the amount of virus in them was strongly related to the state of an epidemic in the population. A recent study conducted in Paris confirmed that the increase in COVID-19 cases is accurately impacted by the increase of genome units in untreated wastewater. This observation was very similar to another study in the Murcia Region (Spain). The results showed a close relationship between the prevalence of viruses and the load of viruses in the WWTP influents [29,143]. Therefore, the issue is expected to significantly influence treated wastewater when wastewater viral loads during a pandemic outbreak are higher than expected.

Consequently, the influent of high viral load cause inefficiency in the virus reduction of effluent before discharge. Thus, wastewater treatment facilities would be required quick responses to minimize the risk of spreading infection during outbreaks since remain viruses in wastewater effluents would perhaps affect recreation, irrigation, and drinking waters [183,184].

Of note, this issue can become one of the main challenging issues that most developing countries face now because they could not be easily equipped with the advanced wastewater treatment technologies regarding inadequate infrastructure compared with developed countries. Accordingly, modern wastewater treatment plants significantly protect public health [151,182]. For example, the results acquired in case of sampling and analysis from the WWTPs effluents in the United Arab Emirates demonstrated that none of wastewater effluent samples tested positive for SARS-CoV-2 RNA because of implementing a sequence of treatment technologies inclusive of preliminary, primary,

secondary (activated sludge process/clarification), and tertiary (sand filtration, disinfection, chlorination). Indeed, these advanced technologies allow them to provide the safety of reusing treated wastewater for irrigating agricultural lands and urban green spaces [165].

Currently, most of the recently published data on SARS-CoV-2 only exist from detection in human stool and municipal wastewater. Nevertheless, the existence of SARS-CoV-2 RNA in the effluents of wastewater treatment plants and at various stages in the wastewater treatment plants has been analyzed by limited studies (Table S4).

Hence, the presence of SARS-CoV-2 RNA in treated wastewater raises a new question that the contaminated effluents of wastewater treatment plants can potentially act as an indirect route of transmission for COVID-19. At present, no evidence has conclusively revealed the infection spread of SARS-CoV-2 through public exposure to contaminated outlets of WWTPs even. However, the number of studies across various countries in the Middle East like Iran (in Tehran, Anzali, and Qom: cities by high population density and outbreak of COVID-19) as well as European countries like French (in Paris) have indicated the presence of SARS-CoV-2 RNA in samples from treated municipal wastewater during the coronavirus disease 2019 outbreak [151,185].

6.1. Dissemination of SARS-CoV-2 from treated municipal wastewater for irrigation

Over the past decades, treated wastewater, owing to inadequate water resources, is most widely used to irrigate agriculture fields and the cities' green areas in numerous countries. Presently, in developing countries (such as Pakistan, India, Mexico, Iran, etc.), it is ubiquitous in reusing approximately 80% of untreated or partially treated domestic wastewater for irrigation. Accessing sufficient water quality for irrigation is essential to slow the spread of the disease and protect people's health. According to published papers (Table S4), the operation of present wastewater treatment plants, like conventional activated sludge, on the inactivation of SARS-CoV-2 RNA has been studied. Current work has also shown that SARS-CoV-2 RNA was detected positive in samples of raw and treated wastewater like in Paris (France), North-Rhine Westphalia (Germany), Tehran (Iran), Gujarat (India), and Yamanashi Prefecture (Japan). Based on current investigations, the current biological wastewater treatment process seems to be reliable to remove SARS-CoV-2 RNA when equipped with appropriate disinfection systems. In other words, even though SARS-CoV-2 RNA in the wastewater can be decreased or even totally remove, few findings have approved the presence of SARS-CoV-2 RNA in WWTP effluents. This can present a serious threat to societies in which untreated or inadequately treated wastewater are used for irrigation of agricultural lands and watering green spaces. Although the guidelines for unrestricted use of domestic wastewater in agriculture irrigation have been defined by the World Health Organization, reaching these standards is difficult in many developing countries. In a better word, sprinkler/spray of treated wastewater can lead to the pathogens that exist in the wastewater and become aerosolized during irrigation. Importantly, viruses have the most tremendous infectious among different pathogens found in wastewater. As a matter of fact, the generated aerosols of irrigation treated wastewater may pose a significant health risk for the irrigators and people near areas irrigated with wastewater because they are likely to cause infection if inhaled [2,54,93,131,186].

Besides the irrigation system, viruses and other pathogens in treated wastewater can be deposited on the soil surface and the surface of leaves or other tissues by irrigation. Then, windy periods can cause the aerosolization of pathogens from the surface of soil and plants. For the first time, Girardin et al. reported that 1–15% of viruses conveyed to the soil during irrigation were converted into airborne aerosols, of which 11–89% become aerosols during the first half-hour [93,187–189].

Furthermore, in another study, Courault et al. indicated the results of a study conducted on the concentration of aerosolized viruses from reclamation of wastewater for irrigation, regarding distance and wind

speed, in France. The health risk assessment of airborne enteric viruses showed a tremendous increase in infection risk with the decreasing distance from the source of the emissions and the rising wind speed. Hence, the dispersion of these aerosols on regional scales can expose farm workers, and people located close to areas irrigated with reuse wastewater and high infection levels [187,190]. Undoubtedly, the possibility of SARS-CoV-2 transmission via exposure pathways linked with agricultural reuse of wastewater needs a crucial consideration and requires further research during a current outbreak.

7. Social effects of SARS-CoV-2

The current pandemic has had repercussions for the whole social community. The media's coverage of the soaring number of mortality, as well as public limitations and quarantine, is likely to exacerbate anxiety, which might have significant consequences for social and mental health worldwide [191]. For instance, it has been reported that the COVID-19 pandemic caused dread in half of the overall population, although it generally had a stressful light effect [192]. Another report by Junling et al. showed a 48% prevalence of sadness and anxiety among the Chinese populace, which was linked to social media usage [193]. Similarly, due to the outbreak, up to 38% of people were forced to work from home. A quarter of the people quit working, and their mental and physical health suffered as a result. Physically active people were more susceptible to mental health problems [194]. Also, Psychological discomfort was reported by up to 35% of those under lockdown. It has been demonstrated that young people, women, high-educated people, and the elderly were considerably more likely to acquire Post-traumatic stress disorder (PTSD) [195]. In health care settings, anxiety was mentioned by 23.2% of medical professionals, insomnia by 38.9%, and depression by 22.8%. Emotional symptoms were more common in female workers and nurses [196]. Furthermore, a recent study by Kang et al. showed that 22.4% of medical and nursing personnel in Wuhan had mild and 6.2% severe disruptions in the aftermath of the pandemic, particularly among young women. A total of 17.5% were required to attend counseling or psychotherapy [197].

8. Further research

For nearly more than one year, the world has got involved in the pandemic of the COVID-19 outbreak. Scientists worldwide study how to combat (identification, probable transmission routes, and disinfection) this disease. Hence, a mass of data is being produced every day. One of the main issues that have garnered worldwide attention at this crucial moment is the spread of SARS-CoV-2 through environmental media.

Several pathways of SARS-CoV-2 dissemination have been known, including inhalation, aerosol, and human contact. This review provided a summary of the probable transmission route of SARS-CoV-2 via aerosols in WWTPs and toilets. The health risks of aerosolization of SARS-CoV-2 are more significant than scientists expected. Thus, the transmission route of COVID-19 via aerosol in WWTPs and toilets must be more investigated. Also, the survival of SARS-CoV-2 RNA in wastewater and the influential factors for the survival of SARS-CoV-2 RNA in wastewater were discussed. In the meantime, it can be helpful that the governments invest in monitoring wastewater to estimate the extent of the current pandemic or any other ones in the future.

As presented, bioaerosols carry viruses, pathogens, and pathogens. The review demonstrates the bioaerosols generation in WWTPs and toilets. Based on results, the number of bioaerosols in toilets depends on toilet types, the energy of flushing, and distances from the toilet. Based on recent studies, there are some measurements to limit the transmission of viruses via bioaerosols. Providing a distinct wastewater collection network for feces and vomits of patients is suggested to prevent bioaerosol generation. Also, closing the toilet lid before flushing and using the extractor fan effectively reduces airborne pathogens in the bathroom. Moreover, consider a disinfection system before flushing can

remove the bioaerosols effectively.

Furthermore, the bioaerosols generated in WWTPs contain toxic compounds of pathogens that are harmful to personnel there. In the current pandemic, the infected people enter the mass of viral loads in wastewater that threaten transmitting the disease. This issue will be even worse in high population density areas or in some regions that lack a suitable treatment system. Knowing the factors that influence the survival and spread of COVID-19 in wastewater could help prevent the spread of this disease. Through the article, it mentioned that WWTP bioaerosols mainly generate in units that are accompanied by turbulence. So the state of the art technologies should be used to decrease agitation. Besides, it can be considered in units with high agitation an automatic system instead of workers to reduce the infectious probability. Above all, the following recommendations are based on research gaps identified in research studies:

1. The concentration of SARS-CoV-2 spreading by airborne bioaerosol in an aura of ambiguity, and there is no evidence of the extent of the virus in environments like agriculture.
2. Investigation on fecal bioaerosol as a route of SARS-CoV-2 transmission
3. Information on toilet plume aerosols generated by toilet flushing
4. The possibility of SARS-CoV-2 transmission via pathways linked with agricultural reuse.

9. Conclusion

This review focuses on the transmission of aerosolized SARS-CoV-2 RNA in toilet flushing, the plumbing system of houses, wastewater treatment plants, and wastewater reuse for irrigation. Based on little evidence, the SAR-CoV-2 transmission via bioaerosols in wastewater was expected to require more studies. The existence and persistency of SARS-CoV-2 RNA in raw wastewater have been reported in recent research. It was found that aerosols play a crucial role in the transmission of SARS-CoV-2. Specifically, wastewater treatment plant workers, farmers, and people who use public toilets are at high risk. Moreover, results from the quantitative microbial risk assessment for toilet flushing, faulty drain, and workers of wastewater treatment plants presented that these places are vulnerable to transmitting the SARS-CoV-2 RNA, which needs further research. This review helps society to have a great awareness about the SARS-CoV-2 transmission in the environment's surroundings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors would like to express their gratitude to the School of Environment, College of Engineering, University of Tehran, Tehran, Iran for supporting this study.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jece.2021.106201](https://doi.org/10.1016/j.jece.2021.106201).

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